REMARKS

Claims 1, 4, 7, 14, 57 and 58 are pending. Claim 1 has been amended with the subject matter of canceled Claim 7. No new matter is added.

The applicant appreciates the Examiner's time to discuss this application in an interview.

Claims 1, 4 and 57 are rejected under 35 U.S.C. §103(a) as being unpatentable over Mejia et al. (Genomics 70(2): 165 – 170, 2000) in view of Perkins et al. (US 2003/0119104A1), Waye et al. (Mol and Cell. Biol. 6(9):3156-3165, 1986), Ikeno et al. (Human Mol. Gen 3(8):1245 – 1257, 1994), Ikeno et al. (Nature Biotech. 16: 431 – 439, 1998) and Bigger et al. (J. Biol. Chem. 276 (25):23018 – 23027, 2001). (Office Action, p. 4).

Claim 1 is amended with the subject matter of claim 7 making this rejection now moot.

Claim 7 is rejected under 35 U.S.C. §103(a) as being unpatentable over Mejia et al. (Genomics, 2000) in further view of Waye et al. (Mol and Cell. Biol., 1986), Ikeno et al. (Human Mol. Gen, 1994), Perkins et al. (US 2003/0119104 A1), Ikeno et al. (Nature Biotech., 1998) and Bigger et al. (J. Biol. Chem. 276 (25):23018 – 23027, 2001), as applied to claims 1, 4 and 57 above, and in further view of Bokkelen et al. (US Patent No. 5,695,967). (Office Action, p. 6).

Mejia is cited as the primary reference, in combination with Waye, Ikeno (1994), Perkins, Ikeno (1998), Bigger and Bokkelen.

The rejection asserts that Mejia et al. teach that the Cre-mediated method may be used to integrate at least two genomic inserts into a first vector, and Ikeno et al. taught the step of selecting from transformed cells a cell containing a specific mammalian artificial chromosome (MAC) having at least two copies of the first vector and/or at least two copies of the second vector. The rejection alleges that Ikeno et al. taught that a vector comprising a mammalian centromere sequence comprising an 11-mer repeat obtained from human chromosome 21 can naturally multimerize by recombination or amplification when producing a MAC in a eukaryotic host cell via homologous recombination. The rejection argues that the Bigger reference teaches the formation of high-molecular multimers by homologous recombination between circular molecules flows from the activity of Cre recombinasae.

It is now claimed that in the first step, the size of the mammalian centromere sequence is about 50 kb or less. This is not disclosed or not at all obvious from the cited art as will be shown herein.

The mammalian centromere sequence being about 50 kb or less is the size of the mammalian centromere sequence comprised in the first vector which is used in the first step. This feature dramatically improves efficiency as supported by the data in Example 2 of the application (specification p.33-34), which shows the difference of efficiency between the case where 100 kb (CMV/α100) alphoid fragment was used (1 out of 16 (6%)) and the case where 50 kb (SV/α50) alphoid fragment was used (3 out of 17 (18%)) (specification p.34). Considering a report by Yasuhide Okamoto et al., which reports no difference in efficiency of HAC formation was observed between 50 kb alphoid DNA(33%) and 70 kb alphoid DNA (35%), this data is beyond the expectation of a person ordinarily skilled in the art. The benefit, which was revealed by the work by the present inventors, by using a small-sized centromere sequence as recited in the amended claim is specific to the present method in which a sequence of interest or an insertion sequence therefor is incorporated during the formation of MAC. The use of a small-sized centromere sequence also facilitates operations such as separation, purification of the first vector including the centromere sequence, and furthermore reduces the probability of exfoliation and modification, which possibly occur at the time of cloning and/or proliferation. The application at page 18, second full paragraph states:

> Preferably, the mammalian centromere sequence of the present invention includes a plurality of such alphoid 11mer repeat units. A sequence isolated from the alphoid region of the human chromosome 21 so as to be identified is shown by SEQ ID NO: 3 (about 25 kb alphoid fragment).

The Office Action on p.5 asserts that selecting a cell containing a specific MAC is motivated in part "because Ikeno et al. (1994) taught that human chromosome 21 alphoid arrays [repeat units of about 1.5kb comprising the 11-mer] naturally exist in higher order structures in mammalian chromosomes, i.e. 1.3Mb (500Kb + 480Kb + 20Kb + 330Kb) (Figure 4)."

However this assertion is not correct. Ikeno merely reports that the size of the alphoid array, which is composed of 11mer repeats, was 1.3Mb. 20Kb was the size of a *Pst*I fragment which was detected as a part of the alphoid array in the course of mapping and identifying the

alphoid array. Ikeno does not indicate that 20kb fragment may exist as a functional unit in itself, let alone availability nor utility thereof.

Thus, claiming a small-sized (i.e. "about 50kb or less") centromere sequence is novel and beyond the expectation of a person skilled in the art in consideration of the common knowledge.

The other cited references Mejia, Perkins, Waye Ikeno (1994), Ikeno (1998), Bigger and Bokkelen in combination do not disclose or suggest the method now claimed. It is respectfully requested that the rejection be reconsidered and withdrawn.

Claim 14 is rejected under 35 U.S.C. §103(a) as being unpatentable over Mejia et al. (Genomics, 2000) in further view of Waye et al. (Mol and Cell. Biol., 1986), Ikeno et al. (Human Mol. Gen, 1994), Perkins et al. (US 2003/0119104 A1), Ikeno et al. (Nature Biotech., 1998), Bigger et al. (J. Biol. Chem., 2001) and Bokkelen et al., as applied to claims 1, 4, 7 and 57 above, and in further view of Cooke et al. (WO 00/18941). (Office Action, p. 6).

Claim 1 is amended with the subject matter of claim 7 making this rejection of Claim 14, which depends from Claim 1, now moot.

Claim 58 is rejected under 35 U.S.C. §103(a) as being unpatentable over Mejia et al. (Genomics, 2000) in further view of Waye et al. (Mol and Cell. Biol., 1986), Ikeno et al. (Human Mol. Gen, 1994), Perkins et al. (US 2003/0119104 A1), Ikeno et al. (Nature Biotech., 1998), Bigger et al. (J. Biol. Chem., 2001), Bokkelen et al. and Cooke et al., as applied to claims 1, 4, 7, 57 and 58 above, and in further view of Okazaki et al. (WO 98/08964). (Office Action, p. 6).

Claim 1 is amended with the subject matter of claim 7 making this rejection of Claim 14, which depends from Claim 1, now moot.

In view of the above amendment, applicant believes the pending application is in condition for allowance.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 04-1105.

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Respectfully submitted,

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